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Cervicovaginal Synthesis of IgG Antibodies to the Immunodominant 175-199 Domain of the Surface Glycoprotein gp46 of Human T-Cell Leukemia Virus Type I

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Paired sera, saliva and cervicovaginal secretions from 17 HTLV-I-infected women (19–75 yr) were tested for total IgA and IgG, for IgA and IgG to the immunodominant region gp46/175-Pro-199, for serum IgG to the neutralizing domains gp46/190-Pro-199 and gp46/190-Ser-199, or for *tax-rex* proviral HTLV-DNA. Serum antibodies to gp46/175-Pro-199 were detected more frequently in the IgG (13/17) than in the IgA (5/17) isotypes. The majority (8/12) of anti-gp46/175-Pro-199-positive sera reacted also to gp46/190-Pro-199 or to gp46/190-Ser-199, demonstrating their neutralizing properties. In saliva, antibodies to gp46/175-Pro-199 were not generally detected. In cervicovaginal secretions, IgG to gp46/175-Pro-199, but not IgA, were detected in 6/15 (40%) patients. The mean specific activity of IgG to gp46/175-Pro-199 showed a trend to be higher in cervicovaginal secretions (218 ± 109) than in sera (14 ± 4). Furthermore, in all patients with cervicovaginal IgG to gp46/175-Pro-199, the cervicovaginal/serum ratio (19 ± 6) of anti-gp46 IgG specific activities were markedly above 1. HTLV-DNA was detected in 4/17 salivas, and in 3/15 cervicovaginal secretions, all from patients demonstrating cervicovaginal synthesis of IgG to gp46/175-Pro-199. In conclusion, IgG to gp46/175-Pro-199 in cervicovaginal secretions, when present, appear to be produced primarily locally because of local HTLV-I excretion. Since anti-gp46/175-Pro-199 antibodies usually support reactivities to neutralizing domains, their presence could be relevant for limiting HTLV-I transmission via cervicovaginal secretions.

KEY WORDS: HTLV-I, local immunity, saliva, cervicovaginal secretions, neutralization

INTRODUCTION

Human T-cell leukemia/lymphotropic virus type I (HTLV-I) is primarily transmitted through mucosae, by breast feeding and sexual intercourse [Hino et al., 1985; Höllsberg and Hafler, 1993]. The epidemiologic observations point to a seemingly low efficiency of heterosexual HTLV transmission [Bartholomew et al., 1987; Murphy et al., 1989], most particularly from female-to-male [Kajiyama et al., 1986].

In saliva [Archibald et al., 1987/1988; Bélec et al., 1994; Terada et al., 1994; Hallouin et al., 1995; Yamamoto et al., 1995], as well as in cervicovaginal secretions [Hallouin et al., 1995; Bélec et al., 1996] from HTLV-I-infected patients, HTLV-I-specific antibodies, mainly of the IgG and less frequently of the IgA isotypes, have been identified, suggesting that HTLV is able to elicit a mucosal immune response. Furthermore, salivary HTLV-I-specific antibodies of the IgG isotype possess neutralizing properties, which could contribute to inhibit the cell-to-cell transmission of the virus present in the oral cavity, and finally the putative transmission of HTLV-I via saliva [Yamamoto et al., 1995]. HTLV-I-specific antibody responses in other body fluids, such as genital secretions, could be similarly relevant for the pathogenesis of HTLV transmission via mucosal surfaces.

Essential HTLV-I biological functions depend on the surface glycoproteins. We therefore investigated IgA and IgG antibodies to one of the major immunodominant domains of HTLV-I, comprised between the amino acids 175 and 199 of the gp46 [Matsushita et al., 1986; Horal et al., 1991; Edouard et al., 1994a, 1994b], and supporting neutralizable domains [Tanaka et al., 1991; Kuroki et

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al., 1992; Baba et al., 1993], in paired sera, saliva and cervicovaginal secretions from African patients infected by HTLV-I.

SUBJECTS AND METHODS

Patients and Controls

Seventeen HTLV-I-infected patients and 15 healthy, HTLV- and HIV-seronegative women, living in Franceville, Gabon, were recruited after informed consent was obtained, as previously reported [Bélec et al., 1996]. Their sera were considered HTLV-I/II positive, since reactivity was present to both core (p24) and envelope (gp46 or rgp21) proteins according to the criteria recommended by the World Health Organization [1990]; furthermore, the p24 band was equal to or weaker in intensity than the p19 reactivity, demonstrating positivity for HTLV-I. Women with vaginal discharge, genital bleeding or ulceration, or with parodontopathy or gingival inflammation, were excluded.

Serum, Saliva and Cervicovaginal Fluid Samples

Peripheral blood was collected in dry tubes. Dribbled whole saliva was collected without stimulation. Cervicovaginal secretions were collected by a nontraumatic, standardized 60-sec-vaginal washing with 3 ml of lithium chloride (LiCl, Sigma Chemical Co., St Louis, MO, USA)-phosphate buffered saline (PBS, pH 7.2, 150 mmol/L, BioMérieux, Marcy l'Etoile, France), and immediately placed in thawing ice [Bélec et al., 1995b]. The paired samples were centrifuged within 1 hr at 4°C, at 1,500*g* for 10 min. The sera and the supernatants of the saliva samples and the cervicovaginal secretions were kept frozen at −30°C until processing.

Evaluation of the Dilution Factor Introduced by the Vaginal Washing

The lithium concentrations in the washing buffer Li-PBS ($[Li]_1$) and in the vaginal washing ($[Li]_2$) were evaluated by a flame absorption spectrophotometer. The dilution factor introduced by the washing procedure was estimated by the ratio $DF = [Li]_1 - [Li]_2/[Li]_1$ [Bélec et al., 1995b].

Estimation of Total IgA and IgG

Concentrations of IgG and IgA in sera, saliva and cervicovaginal secretions were measured by an asymmetric sandwich ELISA, using polyclonal anti-human α-chain or γ-chain (Dakopatts, Globstrup, Denmark) as conjugates, and normal serum (Standard-Human-Serum, Behringswerke AG, Marburg, Germany), as standard, as previously described [Lu et al., 1993; Bélec et al., 1995a].

Detection of Antibodies to HTLV-I/gp46

Anti-gp46 antibodies detection was carried out in paired sera, saliva and cervicovaginal washings from all HTLV-I-infected women by a domestic indirect ELISA using different purified synthetic peptide-coated plates.

The gp46 antigen consisted mainly of a 25-mer 175-Pro-199 peptide, corresponding to the sequence of the prototype ATK [Seiki et al., 1983], i.e. with a proline at position 192, which supports immunodominant and/or neutralizable domains. In fact, the gp46/190-199 sequence contains a continuous conformational epitope recognized by the neutralizing rat monoclonal antibody LAT 27 [Tanaka et al., 1991]. We further evaluated the neutralizing properties of anti-gp46/175-Pro-199 positive sera by testing them against one major neutralizable domain of the gp46/190-199 region. Because of HTLV-I antigenic variants in gp46, two internal 10-mer peptides were used [Edouard et al., 1994b]: the gp46/190-Pro-199 peptide, with a proline at position 192, corresponding to the sequence of the prototype AKT [Seiki et al., 1983], and the gp46/190-Ser-199 peptide, with a serine at position 192, corresponding to the Caribbean HS35 prototype [Malik et al., 1988].

The assay procedure was similar to that described previously by Edouard et al. [1994b]. Wells of polyvinyl chloride microtiter plates (Nunc, Kampstrup, Denmark) were coated overnight at 4°C with 100 µl of an optimal concentration of the synthetic peptides, using carbonate bicarbonate buffer (pH 9.6) as coating buffer. The wells were then saturated with 2% bovine serum albumin for 1 hr at 37°C.

For testing, samples were diluted in PBS with PBS-Tween 20 (0.05% vol/vol) at different working dilutions (d). The serum samples were serially twofold diluted; the salivas were diluted at 1:10 and 1:20 for IgA, and at 1:10 and 1:100 for IgG; the cervicovaginal washings at 1:10 and 1:20 for IgA and IgG. One hundred microliters of diluted samples was incubated for 2 hr at 37°C. These dilutions had been previously determined in order to obtain a signal approximately proportional to the concentration of antibodies catched on the well, i.e. between 0.15 and 1.25 OD units. After five washings with PBS-Tween 20, 100 µl of horseradish peroxidase (HRP)-labeled anti-human α-chain polyclonal antibodies (Sigma) or HRP-labeled anti-human γ-chain polyclonal antibodies (Sigma), diluted 1:1,000, were added for 45 min at 37°C. Enzymatic activities were measured with ABTS as substrate, by a plate reader at 490 nm.

For each detected isotype and each tested dilution, the cutoff values for positivity were two times the mean absorbance obtained with the paired sera, salivas and cervicovaginal secretions from HTLV-negative controls.

Specific Activities for Anti-gp46/175-Pro-199 Antibodies

The specific activities (A) of IgA to gp46/175-Pro-199 in paired sera, salivas and cervicovaginal secretions were evaluated at the first dilution (d') giving an OD between 0.15 and 1.25 in 1 µg of serum, salivary or cervicovaginal total IgA respectively, according to the formula $A = OD \times d'/[IgA]_{\mu g/ml}$. For the cervicovaginal secretions, d' was equal to the ratio d/DF.

The specific activities of IgG to gp46/175-Pro-199 in paired sera, salivas and cervicovaginal secretions per µg of serum, salivary or cervicovaginal total IgG respectively, were calculated similarly.

Comparative Avidities of IgG to Whole HTLV-I Antigens

The avidities of IgG antibodies to HTLV-I in paired sera, salivas and cervicovaginal secretions were compared by the method of Pullen et al. [1986], with slight modifications. ELISA plates coated with whole HTLV-I, lysate and rpg21 (Cambridge Biotech Corporation, Worcester, MA, USA) were incubated in duplicate during 90 min at 37°C with a single dilution of each sample (100 μl), chosen to give an absorbance reading with the anti-γ labeled antibodies at about 50% of the maximum for each serum, saliva and cervicovaginal secretions. After three washings with PBS-Tween 20, 100 μl of appropriate molarities of anhydre sodium thiocyanate (Sigma) in PBS were added in each well for 45 min at room temperature. After five further washings, the usual procedure ELISA was carried out for IgG. The ratios of the absorbance value at a defined concentration of thiocyanate out of the absorbance value of the control without thiocyanate (OD/OD_{max}) were plotted.

Detection of Proviral HTLV DNA

The DNA was extracted from 100 μl of mixed saliva (supernatant), and from the whole cellular pellet of cervicovaginal secretions, using the phenol-chloroform method, and precipitated with ethanol.

Detection of HTLV DNA was carried out in 1 μg of extracted DNA by nested polymerase chain reaction (PCR), in the *tax-rex* genes, using commercially available primers sets (Sorin Biomedica, Saluggia, VC, Italy), and according to the protocol previously described [Ferrante et al., 1993; Bélec et al., 1996].

Statistics

The results are expressed as mean ± standard error. The rank order Wilcoxon test, the Fisher exact test, and the Spearman's correlation test were used for statistical analyses.

RESULTS

Study Population

Among the 17 HTLV-I-infected patients (50 ± 4.6 yr), three were symptomatic clinically (Table I). A 75-year-old woman (patient D) had tetrapyramidal syndrome, with bilateral Hoffmann's and Babinski's signs; her cognitive functions were normal. Patient F suffered from an isolated sicca syndrome. A 56-year-old woman (patient M) fulfilled the criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy; furthermore, she had objective evidence of keratoconjunctivitis sicca and swollen parotidis, highly suggestive of Sjögren's syndrome. The controls (37 ± 3.4 yr) consisted mainly of childbearing-aged women (n = 12) and of three menopausal women. The HTLV-I-infected patients were significantly older than the controls ($P = 0.035$). All patients and controls had satisfactory dental condition, except five patients older than 59 years.

Dilution of Cervicovaginal Fluids

The mean DF introduced by the vaginal washing were similar in patients (0.136 ± 0.012) and in controls (0.121 ± 0.011).

Total IgA and IgG in Paired Samples

The mean levels of serum total IgA and IgG were similar in HTLV-I-infected patients (IgA, 2,240 ± 235 μg/ml; IgG, 29,900 ± 1,400 μg/ml) and in controls. However, hypergammoglobulinemia was found in eight (47%) HTLV-I-infected women, including those with HTLV-I-related symptoms, and in only two (13%) controls ($P < 0.05$).

In HTLV-I-infected patients, the mean levels of salivary IgG were within normal limits (HTLV-I+, 14 ± 1.1 μg/ml; controls, 13 ± 0.1 μg/ml), and those of IgA were significantly decreased by comparison with those of healthy controls (HTLV-I+, 87 ± 23 μg/ml; controls, 212 ± 24 μg/ml; $P < 0.001$). Both patients with a sicca syndrome had high levels of salivary immunoglobulins, with decreased salivary IgA/IgG ratio.

In HTLV-I-infected women, the mean levels of cervicovaginal IgA (42 ± 5.3 μg/ml) and IgG (93 ± 15 μg/ml) with significantly lower than those in controls (IgA, 63 ± 7 μg/ml; IgG, 380 ± 19 μg/ml) (respectively, $P = 0.025$ and $P < 0.001$). The prevalences of women with low total IgA + IgG concentrations in cervicovaginal secretions were significantly higher after 60 years (100%) than before this age (13%) ($P < 0.002$).

Detection of IgA and IgG to HTLV-I/gp46 Synthetic Peptides

In serum from HTLV-I-infected women, antibodies to gp46/175-Pro-199 were detected more frequently in the IgG than in the IgA isotypes [13/17 (77%) vs. 5/17 (30%), $P < 0.02$]. The OD of serum IgG to gp46/175-Pro-199 (at 1:1280) correlated strongly with those of serum IgA to gp46/175-Pro-199 (at 1:100) ($P = 0.003$). The majority (8/12) of sera reacting with the peptide gp46/175-Pro-199 recognized at least one of the two 10-mer peptides gp46/190-Pro-199 or gp46/190-Ser-199. However, in four patients (C, H, I, and L), no serum antibodies to gp46/190-Pro-199 or to gp46/190-Ser-199 were detected, despite significant titers of serum IgG to gp46/175-Pro-199. Patient N had serum IgG against both gp46/190-Pro-199 and gp46/190-Ser-199, in association with a serine at the position 192 (data not shown), suggesting in this patient cross-reactivity in ELISA for anti-gp46/190-Ser-199 IgG to gp46/190-Pro-199.

In saliva from HTLV-I-infected women, IgA as well as IgG antibodies to gp46/175-Pro-199 appeared infrequent: only patient O had low level of salivary IgG to gp46/175-Pro-199.

In cervicovaginal secretions from HTLV-I-infected women, IgA to gp46/175-Pro-199 could not be evidenced. By contrast, IgG to gp46/175-Pro-199 were detected in six (40%) out of the 15 evaluated women. All these women had also detectable IgG (6/6) or IgA (5/6) to gp46/175-Pro-199 in their sera. The OD of serum IgG to gp46/

TABLE I. Clinical and Biological Findings in 17 HTLV-I-Infected Women.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Age (years)	25	30	64	75	60	62	65	70	19	67	70	46	56	39	30	30	72
HTLV-I-related symptoms	−	−	−	+	−	+	−	−	−	−	−	−	+	−	−	−	−
Serum IgG to gp46/175-Pro-199 (titer)	1000	0	100	5000	0	640	0	480	2560	320	7680	120	4300	2600	3840	640	0
Antibodies to gp46/175-Pro-199																	
Serum IgG	+	−	+	+	−	+	−	+	+	+	+	+	+	+	+	+	−
Serum IgA	+	−	−	+	−	−	−	−	+	−	+	−	+	−	−	−	−
Saliva IgG	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Saliva IgA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
CVS IgG	+	−	−	+	−	−	−	−	+	−	+	−	+	+	−	nd	nd
CVS IgA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	nd	nd
Antibodies to gp46/190-Pro-199																	
Serum IgG	+	−	−	+	−	−	−	−	−	nd	−	−	−	+	+	−	−
Antibodies to gp46/190-Ser-199																	
Serum IgG	−	−	−	−	−	+	−	−	−	nd	+	−	+	+	−	+	−
Specific activities of antibodies to gp46/175-Pro-199																	
Serum IgG	20	0	1	36	0	9	0	4	38	2	24	1	21	19	53	9	0
Serum IgA	7	0	0	19	0	0	0	0	41	0	23	0	5	0	0	0	0
Saliva IgG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0
Saliva IgA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CVS IgG	323	0	0	1627	0	0	0	0	280	0	513	0	236	293	0	nd	nd
CVS IgA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	nd	nd
HTLV-I tax-rex nucleic acid																	
Saliva	+	−	−	−	−	+	−	−	+	−	−	−	+	−	−	−	−
CVS (cellular pellet)	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	nd	nd

CVS: cervicovaginal secretions; nd: not done; −: negative; +: positive.

175-Pro-199 (at 1:1280) correlated strongly with those of cervicovaginal IgG to gp46/175-Pro-199 (at 1:10 dilution of the cervicovaginal fluids) ($P = 0.005$).

Relative Avidity of Anti-HTLV IgG

In ELISA, absorbance levels depend both on the specific antibody concentration and on the antibody affinity of the antigen used. Since differences in avidities of IgG to HTLV-I between sera and cervicovaginal secretions could induce variations of the results obtained by the assay, the relative avidity indexes of IgG were evaluated in two selected paired sera, salivas and cervicovaginal secretions. Comparative investigation of the functional avidity of IgG antibodies to HTLV-I by thiocyanate dissociation in patients A and I showed identical curves for sera, salivas and cervicovaginal secretions (not shown). These features demonstrate a similar avidity of the anti-HTLV-I-specific IgG in sera, salivas and in cervicovaginal secretions for the whole HTLV-I antigens. We can thus assume that differences observed in immunoassays between results in sera, saliva and cervicovaginal secretions should a priori not be related to differences in the relative avidities of the antigens used, but to differences in the levels or in the proportions, of anti-HTLV-I IgG antibodies.

Specific Activities for IgA and IgG to gp46/175-Pro-199

The specific activities of IgA and IgG to gp46/175-Pro-199 in saliva and of IgA to gp46/175-Pro-199 in cervicovaginal secretions were zero, except in the saliva of patient O (Table I). In this patient, the specific activities of IgG to gp46/175-Pro-199 in serum was slightly higher than that in saliva, suggesting that the salivary anti-gp46 antibodies were likely of transudative origin. The mean specific activity of IgG to gp46/175-Pro-199 was significantly higher in cervicovaginal secretions (218 ± 109) and in sera (14 ± 4) than in salivas (2 ± 1.9) (respectively, $P < 0.014$ and $P < 0.001$).

Interestingly, the mean specific activity of IgG to gp46/175-Pro-199 showed a trend to be higher in cervicovaginal secretions than in sera, although the difference did not reach statistical significance because of large interindividual variations ($P < 0.07$). However, in all patients with detectable cervicovaginal IgG to gp46/175-Pro-199, the cervicovaginal/serum ratio (19 ± 6) of anti-gp46 IgG specific activities were above 1; between cervicovaginal secretions and sera, the ratio of the specific activities in the same weight of IgG stood between 10 and 45. These features strongly suggest that antibodies to HTLV-I/gp46/175-Pro-199 of the IgG isotype in cervicovaginal secretions, when present, are primarily locally produced.

Detection of HTLV-I Proviral *tax-rex* DNA

HTLV nucleic acid was detected in four (24%) salivas and in three (20%) cervicovaginal secretions (Table I and Fig. 1). The detection of DNA provirus in cervicovaginal secretions was constantly associated with a cervicovaginal synthesis of IgG antibodies to gp46/175-Pro-199.

DISCUSSION

In present series, a genital synthesis of antibodies to the immunodominant domain gp46/175-Pro-199 of the IgG isotype was demonstrated in more than one-third

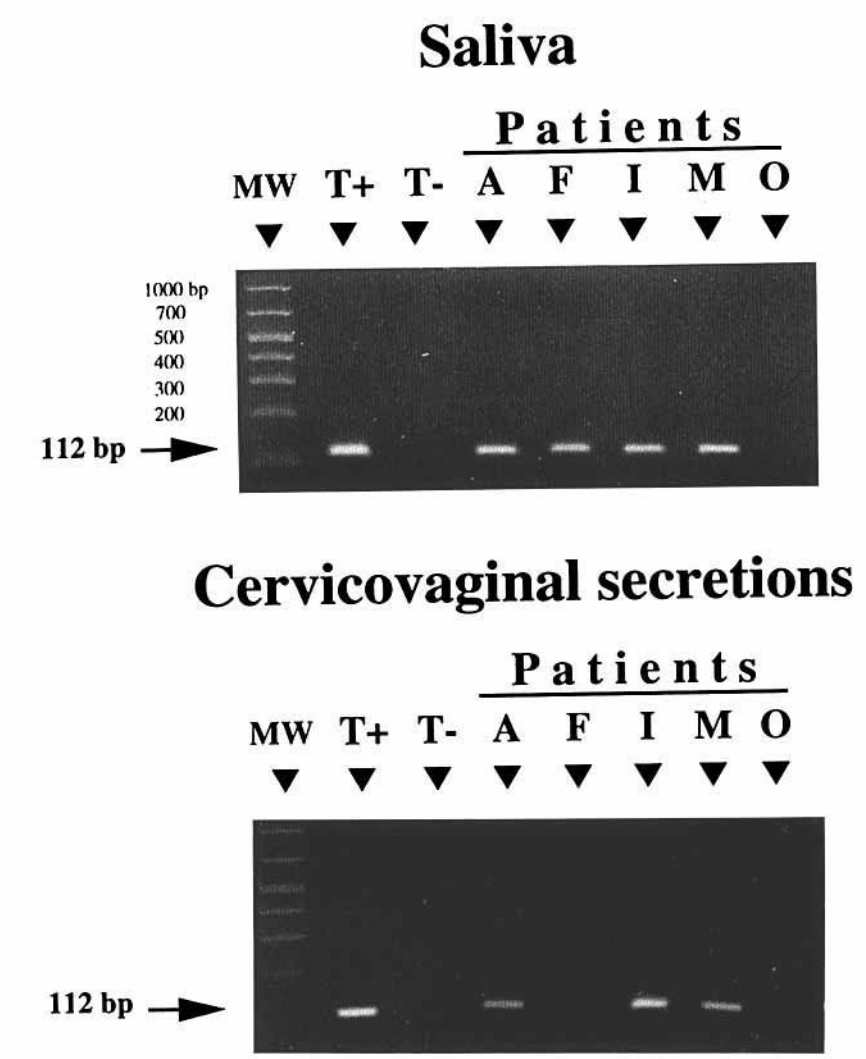

Fig. 1. Detection of *tax-rex* proviral DNA in clinical specimen by nested-PCR. The amplified products were subjected to electrophoresis on a 2% agarose/ethidium bromide gel. Lane 1: Molecular weight markers (BioMarker™ Low, BioVentures, Inc, Murfreesboro, TN). Lane 2: T+: DNA extracted from peripheral blood mononuclear cells of an HTLV-I-infected subject (positive control). Lane 3: T−: master mix with distilled water instead of DNA (negative control). Lanes 4–8. Top: saliva from patients A, F, I, M and O. Bottom: cervicovaginal secretions (cellular pellet) from patients A, F, I, M and O. The molecular size of the amplified products with the inner primers for *tax-rex* proviral DNA is indicated on the left (in base pairs).

of HTLV-I-infected women, in association with cervicovaginal HTLV-I excretion in half of these patients. By contrast, the antibody responses directed to gp46/175-Pro-199 were not significant in saliva for both IgA and IgG isotypes, and in cervicovaginal secretions for the IgA isotype. Since anti-gp46/175-Pro-199 antibodies generally display reactivities to neutralizable domains, their presence in cervicovaginal secretions could be relevant for HTLV-I heterosexual transmission.

In HTLV-negative healthy women, various distributions of total IgA and IgG were observed in saliva and in cervicovaginal secretions, suggesting marked differences in humoral immunity between the oral and the cervicovaginal mucosae. Indeed, the highest immunoglobulin concentration was found for the IgA isotype in the saliva, and for the IgG isotype in the cervicovaginal fluids. In healthy humans, the salivary glands and the oral mucosa contain a predominance of IgA plasma cells (85–90%), and a minority of IgM (6–8%) and IgG (5%) plasma cells [Brandtzaeg et al., 1993], which likely explains the relative predominance of salivary IgA production. Furthermore, a limited contribution to the salivary immunity depends also on the crevicular fluid which flows from the crevice between the gum margin and the tooth, and which reflects the constituents of plasma [Mortimer and Parry, 1991]. In the cervicovaginal secretions of healthy women, the highest immunoglobulin concentrations were found for the IgG isotype, as previously reported (review in [Bouvet et al., 1994]). The normal presence of albumin in cervicovaginal secretions strongly suggests that passive transudation of serum-borne immunoglobulins occurs [Bélec et al., 1995a; Hocini et al., 1995], especially for IgG as, probably, for monomeric IgA, which represents the principal form of IgA in blood. Moreover,

the cervicovaginal mucosa is characterized by a predominance of IgA-producing cells (73–79%), and possesses also plasma cells producing IgM (10–14%) and IgG (7–15%) [Brandtzaeg et al., 1993; Kutteh and Mestecky, 1994]; the uterine mucosa contains a high percentage of IgG-containing cells [Brandtzaeg et al., 1993]. These findings are in keeping with a genital elaboration, not only of IgA or IgM, but also of IgG. In addition, the possibility of an active transepithelial transport for IgG, e.g. via Fcγ receptors, has been hypothesized [Bouvet et al., 1994; Hocini et al., 1995]. Finally, in the female reproductive tract immunity is in part dependent from the secretory immune system [Kutteh and Mestecky, 1994], and it appears also primarily related to the systemic immune system, both by serum-derived and possibly locally produced IgG [Bouvet et al., 1994; Bélec et al., 1995a].

In the majority of HTLV-infected patients, including eight older than 60 years, the total immunoglobulin levels were decreased in the saliva and/or in the cervicovaginal secretions. Age-related alterations of the mucosal functions of the oral [Parvinen and Larmas, 1982; Wardrop et al., 1989] or cervicovaginal [Waldman et al., 1971] mucosae are most probably in part implicated. However, a possible subclinal influence of HTLV infection itself on the mucosal secretions cannot be excluded, and should be investigated with an age-matched control group.

Detection of HTLV-I-specific salivary IgG and IgA has been reported previously in various populations of HTLV-I-infected patients. Furthermore, salivary HTLV-I-specific neutralizing antibodies of the IgG isotype have been evidenced [Yamamoto et al., 1995]. In the series reported by Archibald and colleagues, 21 (95%) of the 22 anti-HTLV-positive salivas precipitated the envelope precursor gp61 or the glycoprotein gp45 [Archibald et al., 1987/1988]. We therefore evaluated the humoral immune response directed specifically to surface glycoprotein gp46/175-Pro-199, which supports major neutralizing domains of HTLV-I, in paired salivas and cervicovaginal secretions from HTLV-I-infected women. Anti-gp46/175-Pro-199 IgA antibodies could not be detected in body fluids, and anti-gp46/175-Pro-199 IgG antibodies were similarly generally absent in salivas. Interestingly, cervicovaginal IgG antibodies to gp46/175-Pro-199 were demonstrated in 40% of HTLV-I-infected women. We further investigated the origin of these cervicovaginal IgG to gp46/175-Pro-199, by correlating the serum and cervicovaginal levels of IgG to gp46/175-Pro-199, and by comparing the anti-gp46/175-Pro-199 antibodies specific activities in sera and in cervicovaginal secretions, i.e. the proportions of anti-gp46 IgG contained in a same weight of total IgG. The titers of serum IgG to gp46/175-Pro-199 correlated strongly with those of cervicovaginal IgG to gp46/175-Pro-199, suggesting in part a transudative origin of cervicovaginal anti-HTLV-I specific antibodies. The transudation of immunoglobulins from serum to vaginal secretions is a physiologic process [Bouvet et al., 1994], and passive transudation of serum-borne IgG to gp46/175-Pro-199 to the cervicovaginal mucosa is consequently likely. In the present series, the mean specific activity of anti-gp46/175-Pro-199 IgG per μg of total IgG showed a trend to be higher in cervicovaginal fluids than in sera, although not significantly, and the cervicovaginal secretions/serum ratios of anti-gp46/175-Pro-199 IgG specific activities were markedly above 1 in patients with cervicovaginal anti-gp46/175-Pro-199 IgG. These findings strongly suggest a primarily genital origin of cervicovaginal gp46/175-Pro-199-specific IgG. The mucosal synthesis of IgG to gp46 is probably due to the presence of replicative HTLV-I in the cervicovaginal mucosa, which contains antigen-presenting cells and lymphocytes [Brandtzaeg et al., 1993]. The cervicovaginal HTLV-I excretion attested by a positive nested-PCR for *tax-rex* proviral DNA was constantly associated with both detection and local synthesis of cervicovaginal anti-gp46/175-Pro-199 IgG. Lack of evidence of HTLV proviral DNA in other patients demonstrating cervicovaginal synthesis of anti-gp46 IgG could be due either to very low levels of HTLV-I, or to false-negative results by nested-PCR, since the presence of PCR inhibitory factors in body fluids is frequent [Ochert et al., 1994]. Finally, cervicovaginal antibodies to the immunodominant domain gp46/175-Pro-199 are exclusively of the IgG isotype; they originate primarily from a cervicovaginal production, and also in part from serum by passive transudation.

Neutralizing B-cell domains of HTLV-I have been identified in gp46 [Tanaka et al., 1991; Baba et al., 1993; Desgranges et al., 1994; Tanaka et al., 1994], and in gp21 [Desgranges et al., 1994]. Then, antibodies against gp46 envelope glycoproteins of HTLV-I are able to neutralize pseudotypes of vesicular stomatitis virus bearing HTLV-I envelope glycoproteins [Clapham et al., 1984], and to inhibit syncytium formation between HTLV-I-infected and susceptible bearing-cells [Nagy et al., 1983]. Furthermore, there is strong evidence that antibodies to the envelope glycoproteins of HTLV-I have neutralizing capabilities and should constitute protective effectors against HTLV-I infection in vivo. In animal models, the transmission by blood transfusion in rabbits can be prevented experimentally by passive immunization with HTLV-I immune globulins [Kataoka et al., 1990]; cynomolgus monkeys [Nakamura et al., 1987] and rabbits may be protected against HTLV-I infection after injection of recombinant vaccinia viruses containing the entire or a partial C-terminal half of the *env*-glycoproteins [Takehara et al., 1989]. Peptides mimicking neutralizable domains of the gp46 were able to induce neutralization antibody responses in animals, which were protective against primary intravenous cell-associated HTLV-I infection [Tanaka et al., 1994]. Whether HTLV-specific antibodies in cervicovaginal secretions or in other body fluids may have any effect on viral transmission remains to be determined. Salivary HTLV-I-specific neutralizing antibodies of the IgG isotype could contribute to inhibit the cell-to-cell transmission of the virus in the oral cavity [Yamamoto et al., 1995]. Since salivary antibodies to gp46/175-Pro-199 could not be detected in our series, the neutralizing process induced by salivary anti-

HTLV-I antibodies is probably associated with neutralizing domains other than those present in the gp46/190-199 domain. In other respects, it is conceivable that locally produced or passively transudated cervicovaginal IgG antibodies to gp46/175-Pro-199 could remain active at the cervicovaginal level and have a possible effect on sexual transmission. Indeed, the majority of sera reacting with the gp46/175-Pro-199 peptide recognized at least one of the two 10-mer gp46/190-Pro-199 or gp46/190-Ser-199 peptides, suggesting that the detected serum IgG to gp46 also possesses neutralizing properties. We can postulate that the cervicovaginal IgG to gp46/175-Pro-199 similarly possesses neutralizing properties. The possibility of antigenic variants in the immunodominant domain of the gp46/175-Pro-199 is not excluded [Edouard et al., 1994b; Moynet et al., 1995], in four patients, in whom no serum antibodies to gp46/190-Pro-199 or gp46/190-Ser-199 were detected, despite significant titers of serum IgG to gp46/175-Pro-199. In HTLV infection, the rates of female-to-male as well as male-to-female heterosexual transmission of the virus are low in absence of associated sexually transmitted diseases, and HTLV-discordant heterosexual couples are frequently encountered [Kajiyama et al., 1986; Murphy et al., 1989; Riedel et al., 1989]. The presence of HTLV-infected cells in genital secretions is consequently insufficient to result uniformly in transmission to sexual partners. Neutralizing cervicovaginal antibodies to gp46/175-Pro-199 could hamper the viral transmission by the immune exclusion of the major viral domains implicated either in the attachment of HTLV on the surface cell, or in the cell-to-cell fusion. Antibodies of the IgG isotype are thought to be very effective for protection against infectious diseases, including viral infections, by inactivating the inoculum at the mucosal surfaces [Robbins et al., 1995]. In addition, since the gp46/190-199 sequence harbors an antibody-dependent cellular cytotoxicity epitope [Kuroki et al., 1992], cell-mediated immune response against HTLV-I could also occur in the cervicovaginal mucosa from some HTLV-I-infected patients.

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REFERENCES

Archibald D, Essex M, McLane MF, Sauk J, Tachibana N, Mueller N (1987/1988): Antibodies to HTLV-1 in saliva of seropositive individuals from Japan. Viral Immunology 1:241–246.

Baba E, Nakamura M, Tanaka Y, Kuroki M, Itoyama Y, Nakano S, Niho Y (1993): Multiple neutralizing B-cell epitopes of human T-cell leukemia virus type 1 (HTLV-1) identified by human monoclonal antibodies. A basis for the design of an HTLV-1 peptide vaccine. Journal of Immunology 151:1013–1024.

Bartholomew C, Saxinger WC, Clark JW, Gail M, Dudgeon A, Mahabir B, Hull-Drysdale B, Cleghorn F, Gallo RC, Blattner WA (1987): Transmission of HTLV-I and HIV among homosexual men in Trinidad. Journal of the American Medical Association 257:2604–2608.

Bélec L, Georges M-C, Pillot J, Georges AJ (1994): Antibodies to HTLV-I in Sjögren's syndrome. Lancet 245:71–72.

Bélec L, Dupré T, Prazuck T, Tévi-Bénissan C, Kanga J-M, Pathey O, Lu X-S, Pillot J (1995a): Cervicovaginal overproduction of specific IgG to human immunodeficiency virus (HIV) contrasting with normal or impaired local response in HIV infection. Journal of Infectious Diseases 172:691–697.

Bélec L, Meillet, Lévy M, Georges AJ, Tévi-Bénissan C, Pillot J (1995b): Dilution assessment of cervicovaginal secretions obtained by vaginal washing for immunological assays. Clinical and Diagnostic Laboratory Immunology 2:57–61.

Bélec L, Georges AJ, Hallouin M-C, Si Mohamed A, Morand-Joubert L, Georges-Courbot M-C (1996): Human T-lymphotropic virus type I excretion and specific antibody response in paired saliva and cervicovaginal secretions. AIDS Research and Human Retroviruses 12:157–167.

Bouvet J-P, Bélec L, Pirès R, Pillot J (1994): Immunoglobulin G antibodies in human vaginal secretions after parenteral vaccination. Infection and Immunity 62:3957–3961.

Brandtzaeg P, Christiansen E, Müller F, Purvis K (1993): Humoral immune response pattern of human mucosae, including the reproductive tracts. In Griffin PD, Johnson PM (eds): "Local Immunity in Reproductive Tract Tissues." Oxford: Oxford University Press, pp 97–130.

Clapham P, Nagy K, Weiss R (1984): Pseudotypes of human T-cell leukemia virus type I and II: neutralization by patients' sera. Proceedings of the National Academy of Sciences of the United States of America 81:2886–2889.

Desgranges C, Souche S, Vernant J-C, Smadja D, Vahlne A, Horal P (1994): Identification of novel neutralization-inducing regions of the human T cell lymphotropic virus type I envelope glycoproteins with human HTLV-I-seropositive sera. AIDS Research and Human Retroviruses 10:163–173.

Edouard E, Legrand E, Astier-Gin T, Dalibart R, Geoffre S, Dalbon P, Guillemain B, Londos-Gagliardi D (1994a): Characterization of monoclonal antibodies directed against the gp46 of human T-cell leukemia virus type I. Leukemia 8:S60–S64.

Edouard E, Londos-Gagliardi D, Busetta B, Geoffre S, Dalbon P, Moeau J-P, Guillemain B (1994b): Recognition by human sera of a variable region of the surface glycoprotein of HTLV-I. Leukemia 8:S65–S67.

Ferrante P, Colombo E, Magistrelli C, Guerini F, Mendozzi E, Cattaneo E, Achilli G (1993): Utilizzo della PCR e dell'isolamento virale per lo studio delle infezioni da HTLV I e HTLV II. 12° Simposio—Epatite e AIDS, Rimini, pp 222–233.

Hallouin M-C, Bélec L, Georges AJ, Si Mohamed A, Georges M-C (1995): HTLV-I excretion and specific antibody response in paired saliva and cervicovaginal secretions. Poster at the 35th ICAAC, San Francisco, California, USA, 17–20 September 1995. Abstract 1191.

Hino S, Yamaguchi K, Katamine S, Sugiyama H, Amagasaki T, Kinoshita K, Yoshida Y, Doi H, Tsuji Y, Miyamoto T (1985): Mother-to-child transmission of human T-cell leukemia virus type I. Japanese Journal of Cancer Research 76:474–480.

Hocini H, Barra A, Bélec L, Iscaki S, Preud'Homme J-L, Pillot J, Bouvet J-P (1995): Systemic and secretory humoral immunity in the normal human vaginal tract. Scandinavian Journal of Immunology 42:269–274.

Höllsberg P, Hafler DA (1993): Pathogenesis of diseases induced by human lymphotropic virus type I infection. New England Journal of Medicine 328:1173–1182.

Horal P, Hall WW, Svennerholn B, Lycke J, Jeansson S, Rymo L, Kaplan M, Vahlne A (1991): Identification of type-specific linear epitopes in the glycoproteins gp46 and gp21 of human T-cell leukemia viruses type I and type II using synthetic peptides. Proceedings of the National Academy of Sciences of the United States of America 88:5754–5758.

Kajiyama W, Kashiwagi S, Ikematsu H, Hayashi J, Nomura H, Okochi K (1986): Intrafamilial transmission of adult T-cell leukemia virus. Journal of Infectious Diseases 154:851–857.

Kataoka R, Takehara N, Iwahara Y, Sawada T, Ohtsuki Y, Dawei Y, Hoshino H, Miyochi I (1990): Transmission of HTLV-I by blood transfusion and its prevention by passive immunization in rabbits sera. Blood 76:1657–1661.

Kuroki M, Nakamura M, Itoyama Y, Tanaka Y, Shiraki H, Baba E, Esaki T, Tatsumoto T, Nagafuchi S, Nakano S, Niho Y (1992): Identification of new epitopes recognized by monoclonal antibodies with neutralizing and antibody-dependent cellular cytotoxicity activities specific for human T-cell leukemia virus type I. Journal of Immunology 149:940–948.

Kutteh WH, Mestecky J (1994): Secretory immunity in the female

reproductive tract. American Journal of Reproductive Immunology 31:40–46.

Lu X-S, Bélec L, Pillot J (1993): Anti-gp160 IgG and IgA antibodies associated with a large increase in total IgG in cervicovaginal secretions from human immunodeficiency virus type-1 infected women. Journal of Infectious Diseases 167:1189–1192.

Malik KTA, Even J, Karpas A (1988): Molecular cloning and complete nucleotide sequence of an adult T-cell leukemia virus/human T-cell leukemia virus type I (ATLV/HTLV-I) isolate of Caribbean origin: Relationship to other members of the ATLV/HTLV-I subgroup. Journal of General Virology 69:1695–1710.

Matsushita S, Robert-Guroff M, Trepel J, Cossmann J, Mitsuya H, Broder S (1986): Human monoclonal antibody directed against an envelope glycoprotein of human T-cell leukemia virus type I. Proceedings of the National Academy of Sciences of the United States of America 83:2672–2676.

Mortimer PP, Parry JV (1991): Non-invasive virological diagnosis: Are saliva and urine specimens adequate substitutes for blood? Review of Medical Virology 1:73–78.

Moynet D, Cosnefroy J-Y, Bedjabaga I, Roelants G, Georges-Courbot M-C, Guillemain B (1995): Identification of new genetic subtypes of Human T Cell Leukemia Virus Type I in Gabon from encoding sequence of surface envelope glycoprotein. AIDS Research and Human Retroviruses 11:1407–1411.

Murphy EL, Figueroa JP, Gibbs WN, Brathwaite A, Holding-Cobham M, Waters D, Cranston B, Hanchard B, Blattner WA (1989): Sexual transmission of human T-lymphotropic virus type I (HTLV-I). Annals of Internal Medicine 111:555–560.

Nagy K, Chapham P, Cheinsong-Popov R, Weiss R (1983): Human T-cell leukemia virus type I: Induction of syncytia and inhibition by patients' sera. International Journal of Cancer 32:321–328.

Nakamura H, Hayami M, Ohta Y, Ishikawa K, Tsujimoto H, Kiyokawa T, Yoshida M, Sasagawa A, Honjo S (1987): Protection of cynomolgus monkeys against infection by human T-cell leukemia virus type-I by immunization with viral *env* gene product produced in *Escherichia coli*. International Journal of Cancer 40:403–407.

Ochert AS, Boulter AW, Birnbaum W, Johnson NW, Teo CG (1994): Inhibitory effect of salivary fluids on PCR: Potency and removal. PCR Methods and Application 3:365–368.

Parvinen T, Larmas M (1982): Age dependency of stimulated salivary flow rate, pH, and bacillus and yeast concentrations. Journal of Dental Research 61:1052–1055.

Pullen GR, Fitzgerald MG, Hosking CS (1986): Antibody avidity determination by ELISA using thiocyanate elution. Journal of Immunological Methods 86:83–87.

Riedel DA, Evans AS, Saxinger C, Blattner WA (1989): A historical study of human T-lymphotropic virus type I transmission in Barbados. Journal of Infectious Diseases 159:603–609.

Robbins JB, Scneerson R, Szu SC (1995): Perspective: Hypothesis: Serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. Journal of Infectious Diseases 171:1387–1398.

Seiki M, Hattori S, Hirayama Y, Yoshida M (1983): Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proceedings of the National Academy of Sciences of the United States of America 80:3618–3622.

Takehara N, Iwahara Y, Uemura Y, Sawada T, Ohtsuki Y, Iwai H, Hoshino H, Miyoshi I (1989): Effect of immunization on HTLV-I infection in rabbits. International Journal of Cancer 44:332–336.

Tanaka Y, Zeng L, Shiraki H, Shida H, Tozawa H (1991): Identification of a neutralizing epitope on the envelope gp46 antigen of human T cell leukemia virus type 1 and induction of neutralizing antibody by peptide immunization. Journal of Immunology 147:354–360.

Tanaka Y, Tanaka R, Terada E, Koyanagi Y, Miyano-Kurosaki N, Yamamoto N, Baba E, Nakamura M, Shida H (1994): Induction of antibody responses that neutralize human T-cell leukemia virus type I infection in vitro and in vivo by peptide immunization. Journal of Virology 68:6323–6331.

Terada K, Katamine S, Eguchi K, Moriuchi R, Kita M, Shimada H, Yamashita I, Iwata K, Tsuji Y, Nagataki S, Miyamoto T (1994): Prevalence of serum and salivary antibodies to HTLV-I in Sjögren's syndrome. Lancet 344:116–119.

Waldman RH, Cruz JM, Rowe DS (1971): Immunoglobulin levels and antibody to *Candida albicans* in human cervicovaginal secretions. Clinical and Experimental Immunology 10:427–434.

Wardrop RW, Hailes RJ, Burger H, Reade PC (1989): Oral discomfort at menopause. Oral Surgery, Oral Medicine and Oral Pathology 67:535–540.

World Health Organization (1990): Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2, and HTLV-I/-II. Weekly Epidemiological Records 65:281–283.

Yamamoto T, Terada K, Nishida N, Moriuchi R, Shirabe S, Nakamura T, Tsuji Y, Miyamoto T, Katamine S (1995): Inhibitory activity of cell-to-cell transmission of human T-cell lymphotropic virus in vitro: evaluation of saliva as an alternative source of transmission. Journal of Clinical Microbiology 33:1510–1515.